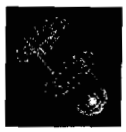

IMMULITE®

Anti-HBc

Total Antibodies to Hepatitis B Core Antigen

DPC®

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IMMULITE® Anti-HBc

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Performance characteristics have not been adequately established for use of the IMMULITE Anti-HBc assay in infants, children, or adolescents.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE Anti-HBc assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use: IMMULITE Anti-HBc is a solid-phase chemiluminescent enzyme immunoassay designed for use on the IMMULITE automated immunoassay analyzer for the qualitative detection of total antibodies against hepatitis B core antigen (anti-HBc) in human serum or plasma (EDTA, heparinized, citrate). It is intended for *in vitro* diagnostic use for the presumptive laboratory diagnosis of ongoing or previous hepatitis B virus infection.

Caution: Performance characteristics for the IMMULITE Anti-HBc were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE Anti-HBc or another legally-marketed anti-HBc assay.

Catalog Number: **LKHC1** (100 tests), **LKHC5** (500 tests)

Test Code: **aBC** Color: **Light Gray**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of Hepadnaviridiae, a DNA virus, and is found world-wide. Distribution of HBV infection will vary among geographical areas and population groups. Transmission of the virus is due to parenteral contact, through the exchange of blood or blood products, sexual contact, and perinatal spread from mother to newborn.[1,2] Clinical manifestations range from mild asymptomatic infections to severe fulminant hepatitis.[1,2,3] Over 90% of infected adults will have an acute self-limiting infection,[2] with approximately 30% of these individuals developing jaundice, liver enzyme elevations, and symptoms. Recovery occurs without any chronic sequelae.[1,2]

Chronic liver disease, a condition in which infection persists for more than six months, a known sequela of a hepatitis B infection, is usually progressive.[1,2] The risk of developing the chronic carrier state is more likely to follow infection acquired in childhood than as an adult.[4,5] In chronic HBV carriers, there is no evidence of continued hepatic damage,[1,2] however, the infection persists and the carrier maintains the ability to transmit the virus.[2]

Availability of HBV vaccines, and the recommendation of universal immunization for infants and other high-risk persons has aided in the prevention of HBV infections. In addition, treatment with alpha-interferon to relieve symptoms is available. Results have shown positive response to treatment in 40–50% of selected individuals with chronic active hepatitis B.[4,5]

Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. The first marker to appear during the incubation phase is HBsAg, and indicates an ongoing infection with HBV.[1,2,4] IgM and total anti-HBc appears shortly after the appearance of HBsAg, when the patient usually becomes symptomatic, and peaks during the acute phase prior to the appearance of anti-HBs. IgM antibody to the core antigen will decline in uncomplicated acute infection, whereas IgG antibody will persist for years.[4,5] Total anti-HBc may also be elevated in chronic HBV infections.[4]

Presence of IgM and total anti-HBc indicates an ongoing or recent HBV infection. These tests can be used in conjunction with other HBV serological

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markers for laboratory diagnosis or to rule out HBV infection.

Principle of the Procedure

IMMULITE Anti-HBc is a solid-phase, two-step chemiluminescent enzyme immunoassay. The solid phase, a polystyrene bead enclosed within a Test Unit, is coated with purified recombinant HBcAg produced in *E. coli* bacteria.

The patient specimen is added to the Test Unit containing a coated bead. An alkaline phosphatase-labeled monoclonal anti-HBc antibody is also added to the Test Unit. After the wash and incubation steps, chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase. IMMULITE Anti-HBc is an antibody competitive assay. The photon output, as measured by the luminometer, is inversely related to the presence of antibodies to HBcAg in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence, the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to falsely elevated results.[9]

The IMMULITE Anti-HBc assay may be performed on human serum or plasma (heparinized, sodium citrate, or EDTA).

If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

Specimens containing precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying. Specimens showing particulate matter or red blood cells may give inconsistent results and should be centrifuged prior to testing (recommended 8,000 to 10,000 RCF × 10 minutes). Specimens which are not tested within 24 hours should be removed from the clot or red blood cells.

Do not use heat-inactivated specimens.

Specimens with obvious microbial contamination should not be tested and recollected.

More than two freeze-thaw cycles are not recommended.

Volume Required: 50 µL serum or plasma (heparinized, sodium citrate or EDTA). (Sample cup must contain at least 100 µL more than the total volume required.)

Storage: 2 days at room temperature (15°–28°C)[10].
3 days at 2–8°C.[6,10]
For longer storage: at –20°C.[7]

Warnings and Precautions

For *in vitro* diagnostic use.

This assay was designed and validated for use with human serum or EDTA, heparin, and Na Citrate plasma from individual patient specimens.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for syphilis; for antibodies to HIV1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

The Anti-HBc Adjustor, Anti-HBc Low Positive and Anti-HBc Positive Controls contain HBcAg which may be indicative of low levels of HBV. These components have been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual,

Biosafety in Microbiological and Biomedical Laboratories, 1993.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

Chemiluminescent Substrate: Avoid contamination and exposure to direct sunlight. (See insert.)

Water: Use distilled or deionized water for the wash step and avoid system contamination.

Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

Anti-HBc Test Units (LHC1)
Each barcode-labeled unit contains one bead coated with purified recombinant HBcAg manufactured at DPC. Stable at 2–8°C until expiration date.
LKHC1: 100 units. **LKHC5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

Anti-HBc Reagent Wedges (LHCA, LHCB)
With barcodes. **LHCA:** 6.5 mL of a protein-based buffer, with preservative. **LHCB:** 6.5 mL alkaline phosphatase (bovine calf intestine) conjugated to purified monoclonal murine anti-HBc in buffer, with < 0.1 g/dL sodium azide. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.
LKHC1: 1 set. **LKHC5:** 5 sets.

Anti-HBc Adjustor (LHCR)
2 mL human serum reactive to HBcAg in a buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.
LKHC1: 1 vial. **LKHC5:** 2 vials.

Anti-HBc Controls (LHCC1, LHCC2, LHCC3) (may be purchased separately.) Three vials containing 2 mL each. **LHCC1 (Negative Control):** human serum nonreactive to HBcAg, with < 0.1 g/dL sodium azide. **LHCC2, LHCC3 (Low Positive Control, Positive Control):** human serum reactive to HBcAg, with <0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.
LKHC1: 1 set. **LKHC5:** 2 sets.

For the current control ratio ranges, please refer to the Control insert.

Kit Components Supplied Separately

LSUBX: Chemiluminescent Substrate
LPWS2: Probe Wash Module
LKPM : Probe Cleaning Kit
LCHx-y: Sample Cup Holders (barcoded)
LSCP: Sample Cups (disposable)
LSCC: Sample Cup Caps (optional)

Also Required
Sample transfer pipets, distilled or deionized water.

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in Section 6 of the IMMULITE Operator's Manual.

See Section 4 of the IMMULITE Operator's Manual for: preparation, setup, adjustment, assay and quality control procedures.

Visually inspect each Test Unit for the presence of a bead before loading it onto the system.

Note that both Reagent Wedges A and B must be loaded on the carousel to run this assay.

Adjustment Interval: 4 weeks. The Anti-HBc Adjustor is used as the cutoff reference to determine the status of a patient specimen.

Quality Control Samples: The controls supplied with the kit should be used as quality control material to monitor assay performance.

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The user should provide alternate control material for a plasma matrix.

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For the current control ratio ranges, please refer to the Control insert.

If control results fall outside the stated range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all patient specimens before reporting results for this run.

The positive control in the test kit is not to be used to quantitate assay sensitivity. The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

It is recommended that the user refer to *NCCLS: Internal Quality Control Testing: Principles and Definitions* and other published guidelines for general quality control recommendations and intervals for testing. [9,11]

Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the test procedure has been strictly adhered to.

Calculation of Cutoff and CO/S Ratio: The Master Cutoff of the assay was determined from representative samples to achieve optimal sensitivity and specificity for the assay.

The cutoff is set equal to the average counts per second of the Adjustor (from the most recent adjustment) multiplied by Curve Parameter 1. (See the "Low Adjustor CPS" and "Curve Parameter 1" fields in the IMMULITE Kit Information screen, which can be accessed from the menu via Data Entry : Kit Entry.)

Calculation of a cutoff/signal (co/s) ratio is done by using the following formula:

$$\text{CO/S Ratio} = \frac{\text{Mean Adjustor cps} \times \text{P1}}{\text{Sample or Control cps}}$$

Calculation and reporting of qualitative results (positive/negative/indeterminate) are handled automatically by the IMMULITE.

The result for a sample is reported as "Indeterminate" if the counts per second for that sample fall within ±15% of the cutoff. The result is reported as "Positive" if the sample's counts are *below* the indeterminate range, and "Negative" if *above* this range.

Interpretation of Results

A result of **"Positive"** (co/s ratio of ≥ 1.15) indicates that anti-HBc antibodies were detected in the sample, which is indicative of either ongoing or previous HBV infection.

A result of **"Negative"** (co/s ratio of < 0.85) indicates that anti-HBc antibodies were not detected in the sample. It is possible the individual is not infected with HBV.

Any result of **"Indeterminate"** (co/s ratio between 0.85 and < 1.15) should be retested. Samples which still test as "Indeterminate" should be tested by an alternate method, or a second sample should be taken — if possible — within a reasonable period of time (e.g., one week).

For the determination of seroconversion, two sera or plasma (heparinized, sodium citrate or EDTA) samples should be drawn three to four weeks apart, during the acute and convalescent stages of the infection.

It is recommended that the following be included with the report to the physician:

> The following results were obtained with the IMMULITE Anti-HBc assay. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported anti-HBc level cannot be correlated to an endpoint titer.

Limitations

IMMULITE Anti-HBc assay is limited to the detection of total anti-HBc in human serum or plasma. The presence of total anti-HBc does not constitute a diagnosis of hepatitis B infection but may be indicative of recent and/or past infection by hepatitis B virus. A nonreactive test result does not exclude the possibility of exposure to hepatitis B virus. Levels of total anti-HBc may be undetectable in early infection or when the patient is asymptomatic.

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Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis and other laboratory tests are nonreactive for the diagnosis of viral hepatitis. The results from this or another diagnostic kit should be used and interpreted only in the context of the overall clinical picture.

A negative result does not indicate that the patient was not infected with HBV. The patient sample should be tested for the presence of other serological markers, such as HBsAg or anti-HBc IgM.

Assay performance characteristics have not been established for any specimen matrices other than serum, or heparinized, EDTA, or sodium citrate plasma.

The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall laboratory and clinical picture. Supportive laboratory and clinical information must be used to determinate the HBV disease state. False results may be obtained with any diagnostic test.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of this type of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other laboratory findings.

Expected Values

Demographics and expected prevalence rates for different categories of subjects, each of whom provided one specimen, from three clinical studies, one in the northwestern United States (Study 1), one in the southern United States (Study 4) and one in Europe, are summarized in the following tables.

Apparently Healthy Subjects (Study 1)

IMMULITE Anti-HBc				
Age	Gender	Total	Pos.	%
0 - 9	Male	0	0	—
	Female	0	0	—
10 - 19	Male	4	1	25.0%
	Female	5	0	0.0%
20 - 29	Male	7	0	0.0%
	Female	13	2	15.4%
30 - 39	Male	2	0	0.0%
	Female	2	0	0.0%
40 - 49	Male	1	0	0.0%
	Female	1	0	0.0%
50 - 59	Male	0	0	—
	Female	0	0	—
60 - 69	Male	0	0	—
	Female	1	0	0.0%
70 - 79	Male	0	0	—
	Female	0	0	—
80 - 89	Male	0	0	—
	Female	0	0	—
90 - 99	Male	0	0	—
	Female	0	0	—
Totals	Male	14	1	7.1%
	Female	22	2	9.1%
	All	36	3	8.3%

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Other subjects

Subject	Total *n*	Male	Female	Mean Age	Age Range	Pos. by IML Anti-HBc	
						n	%
Apparently Healthy (Europe)	1,746	Not available				47	2.7%
Pre-vaccination (Study 1)	17	8	9	34	21-56	0	0.0%
Pregnant at high risk[1] (Study 1)	3	0	3	33	30-36	3	100.0 %
Pregnant at low risk[1] (Study 1)	16	0	16	31	21-42	0	0.0%
Pregnant at low risk[1] (Study 4)	198	0	198	28	17-41	4	2.0%
Pregnant at low risk[1] (Europe)	25	Not available				0	0.0%

[1] At high risk or low risk of exposure to hepatitis B.

Performance Characteristics

I. Clinical Performance

Four clinical studies with 769 specimens and one comparison study with 139 specimens from a total of 908 subjects were conducted to assess the performance of the IMMULITE Anti-HBc assay.

The participating subjects each contributed one specimen to the analyses. The specimens were tested by FDA-approved or licensed hepatitis B assays (Reference markers) and IMMULITE Anti-HBc. All specimens in the analyses were initial test results.

The data were analyzed following the assignment of specimen classification based upon the reactive(+)/ nonreactive(-) patterns for the HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process.

Study 1: Conducted in the northwestern United States, this study included 281 patients, consisting of 138 males and 88 females. Gender for 55 subjects was not reported. These subjects had an average age of 43 years ranging from 21 to 85 years. Distributions of the ethnicity of the subjects are shown in the following table.

Ethnicity	*n*	%
African American	15	5.3%
Caucasian	169	60.1%
Hispanic	2	0.7%
Asian	32	11.4%
Other	3	1.1%
Unknown	60	21.4%
Total	281	100.0%

These subjects included acute and chronic hepatitis B patients, vaccinated individuals, pregnant women, and apparently healthy individuals, and patients with potentially crossreactive substances and medical conditions.

A total of 281 specimens were prospectively (n=92) and retrospectively (n=189) collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 17 unique patterns:

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Characterization based on single point specimens	No. of patients	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	2	+	–	–	–	–	–
Acute	1	+	+	–	–	–	–
Acute	32	+	–	+/-	+	+	–
Acute	34	+	+	+/-	+	–	–
Chronic	2	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	+
Chronic	1	+	–	–	+	–	+
Chronic	1	+	+	+/-	+	–	+
Early Recovery	16	–	–	+/-	+	+	+
Early Recovery	4	–	–	–	+	+	–
Early Recovery	19	–	–	–	+	+/-	–
Early Recovery	1	–	–	+	+	+/-	+
HBV vaccine response	27	–	–	–	–	–	+
Not previously infected	120	–	–	–	–	–	–
Recovered	16	–	–	–	+/-	–	+
Recovered	1	–	+/-	–	+	–	+
Uninterpretable	1	–	+	–	–	–	–

Based on the above classifications the IMMULITE Anti-HBc results were compared to Kit A, a reference assay for the determination of anti-HBc.

Reference	Kit A						Total
	+			–			
	IML Anti-HBc						
	+	Ind	–	+	Ind	–	
HB Acute infection	63	0	3	0	1	2	69
HB Chronic infection	7	0	0	0	0	0	7
HB Early recovery	32	2	6	0	0	0	40
HBV Vaccine response	0	0	0	0	0	27	27
Not previously infected	0	0	0	1	0	119	120
Recovered	12	4	1	0	0	0	17
Uninterpretable	0	0	0	0	0	1	1
Total	114	6	10	1	1	149	281

HB Acute Infection
Positive agreement = 95.5% (63/66)
95% CI = 87.3 to 99.1%
Negative agreement = N/A (2/3)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (7/7)
95% CI = 59.0 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Early Recovery
Positive agreement = 80.0% (32/40)
95% CI = 64.4 to 91.0%
Negative agreement = N/A (0/0)
95% CI = N/A

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (27/27)
95% CI = 87.2 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.2% (119/120)
95% CI = 95.4 to 100.0%

Recovered
Positive agreement = 70.6% (12/17)
95% CI = 44.0 to 89.7%
Negative agreement = N/A (0/0)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total

Positive agreement = 87.7% (114/130)
95% CI = 80.8 to 92.8%
Negative agreement = 98.7% (149/151)
95% CI = 95.3 to 99.8%
Total agreement = 93.6% (263/281)
95% CI = 90.1 to 96.2%

Study 2: Conducted in the northeastern United States, this study included 209 patients, consisting of 104 males, 103 females. Gender for two patients was not reported. These patients had an average age of 47 years, ranging from newborn to 93 years. Distributions of the ethnicity of the patients are shown in the following table.

Ethnicity	*n*	%
African American	21	10.0%
Caucasian	101	48.3%
Hispanic	3	1.4%
Asian	6	2.9%
Other	7	3.3%
Unknown	71	34.0%
Total	209	100.0%

Included were patients with potentially cross-reactive substances and medical conditions.

A total of 209 retrospective specimens were collected and tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated eight unique patterns:

Characterization based on single point specimens	Patient	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Acute	8	+	–	–	–
Acute	9	+	+/-	+	–
Chronic	2	+	–	+	+
Early recovery	33	–	+/-	+	+
Early recovery	17	–	–	+	–
HBV vaccine response	32	–	–	–	+
Not previously infected	107	–	–	–	–
Uninterpretable	1	+	–	–	+

Based on the above classifications the IMMULITE Anti-HBc results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			–			
	IML Anti-HBc						
	+	Ind	–	+	Ind	–	
HB Acute infection	9	0	0	0	0	8	17
HB Chronic infection	2	0	0	0	0	0	2
HB Early recovery	45	2	3	0	0	0	50
HBV Vaccine response	0	0	0	0	0	32	32
Not previously infected	0	0	0	0	1	106	107
Uninterpretable	0	0	0	0	0	1	1
Total	56	2	3	0	1	147	209

HB Acute Infection

Positive agreement = 100.0% (9/9)
95% CI = 66.4 to 100.0%
Negative agreement = 100.0% (8/8)
95% CI = 63.1 to 100.0%

HB Chronic Infection

Positive agreement = N/A (2/2)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

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Early Recovery
Positive agreement = 90.0% (45/50)
95% CI = 78.2 to 96.7%
Negative agreement = N/A (0/0)
95% CI = N/A

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (32/32)
95% CI = 89.1 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.1% (106/107)
95% CI = 94.9 to 100.0%

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = 91.8% (56/61)
95% CI = 81.9 to 97.3%
Negative agreement = 99.3% (147/148)
95% CI = 96.3 to 100.0%
Total agreement = 97.1% (203/209)
95% CI = 93.9 to 98.9%

Study 3: Specimens obtained from China were tested in the southern United States. This study included 79 patients and was comprised of 13 females and 55 males (gender for 11 patients was not reported) with an average age of 36 years, ranging from 18 to 82 years. These were prospectively recruited patients from a clinically well-characterized, homogeneous population: acute hepatitis B patients who presented with symptoms typical of acute hepatitis B such as jaundice, persistent fatigue, loss of appetite, pale stools and liver enlargement. Their ALT and AST results were significantly elevated at the time of diagnosis.

A total of 79 specimens were prospectively collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 11 unique patterns:

Characterization based on single point specimens	n	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	23	+	–	+/-	+	+	–
Acute	8	+	+/-	+	+	+	–
Acute	1	+	–	+	+/-	–	–
Acute	35	+	+	+/-	+	–	–
Acute	4	+	+	+	+	–	+/-
Chronic	1	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	–
Chronic	1	+	+	+/-	+	–	+
Chronic	1	+	+	+	+	+	+
Recovered	1	–	–	–	+/-	–	+
Uninterpretable	1	+	–	+	+	+	+

Based on the above classifications the IMMULITE Anti-HBc results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			–			
	IML Anti-HBc						
	+	Ind	–	+	Ind	–	
HB Acute infection	71	0	0	0	0	0	71
HB Chronic infection	6	0	0	0	0	0	6
Recovered	1	0	0	0	0	0	1
Uninterpretable	1	0	0	0	0	0	1
Total	79	0	0	0	0	0	79

HB Acute Infection
Positive agreement = 100.0% (71/71)
95% CI = 94.9 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (6/6)
95% CI = 54.1 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Recovered
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

Total
Positive agreement = 100.0% (79/79)
95% CI = 95.4 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A
Total agreement = 100.0% (79/79)
95% CI = 95.4 to 100.0%

Study 4: Conducted in the southern United States, this study included retrospectively collected specimens from 200 pregnant subjects. These subjects had an average age of 28 years, ranging from 17 to 41 years.

A total of 200 specimens were tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated three unique patterns:

Characterization based on single point specimens	No. of subjects	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Early recovery	4	–	+/-	+	+
Early recovery	2	–	–	+	–
HBV vaccine response	42	–	–	–	+
Not previously infected	152*	–	–	–	–

Based on the above classifications the IMMULITE Anti-HBc results were compared to Kit A.

Reference	Kit A						Total
	+			–			
	IML Anti-HBc						
	+	Ind	–	+	Ind	–	
Early recovery	4	0	2	0	0	0	6
HBV vaccine response	0	0	0	0	0	42	42
Not previously infected	0	0	0	0	1	149	150*
Total	4	0	2	0	1	191	198

* Two specimens were not tested for IMMULITE Anti HBc.

Early Recovery
Positive agreement = 66.7% (4/6)
95% CI = 22.3 to 95.7%
Negative agreement = N/A (0/0)
95% CI = N/A

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (42/42)
95% CI = 91.6 to 100.0%

Not Previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.3% (149/150)
95% CI = 96.3 to 100.0%

Total
Positive agreement = 66.7% (4/6)
95% CI = 22.3 to 95.7%
Negative agreement = 99.5% (191/192)
95% CI = 97.1 to 100.0%
Total agreement = 98.5% (195/198)
95% CI = 95.6 to 99.7%

Study 5: In an additional study conducted at Diagnostic Products Corporation, IMMULITE Anti-HBc was compared to IMMULITE 2000 Anti-HBc. Presented below are the comparisons between IMMULITE and IMMULITE 2000 Anti-HBc on a total of 139 specimens.

IML Anti-HBc									
+			Ind			–			
IML 2000 Anti-HBc									
+	Ind	–	+	Ind	–	+	Ind	–	Total
78	0	1	0	0	0	0	0	60	139

Positive agreement = 100.0% (78/78)
95% CI = 95.4 to 100.0%
Negative agreement = 98.4% (60/61)
95% CI = 91.2 to 100.0%
Total agreement = 99.3% (138/139)
95% CI = 96.1 to 100.0%

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II. Analytical Performance

See Tables for data *representative* of the assay's performance. Anti-HBc results in the following sections are expressed as a cutoff-to-signal ratio. (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

Analytical Sensitivity: Based on studies with serial dilution of Paul Erlich Institute reference material Anti-HBc IgG 100 U/mL, the analytical sensitivity (last positive dilution) for IMMULITE Anti-HBc is 0.42 PEI U/mL.

The 95% confidence interval at this level (0.42 PEI IU/mL) is 0.37 - 0.48 PEI U/mL.

Precision: Serum samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates at three US sites. The same design was used for three lots and at three sites. (See "Precision" tables.)

EDTA, heparin and sodium citrate samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE Anti-HBc and one lot of IMMULITE 2000 Anti-HBc. The median total variance of coefficients (EDTA, 4.9%; heparin, 4.4%; sodium citrate, 5.5%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 Anti-HBc.

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 20 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 10 and 20 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Hemolysis: Presence of hemoglobin in concentrations up to 504 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 168, 252 and 504 mg/dL of hemoglobin concentrations. Performance was not established with clinical specimens.

Lipemia: Presence of lipemia in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 500, 1000, 2000 and 3000 mg/dL of lipemia triglycerides. Performance was not established with clinical specimens.

Alternate Sample Types: The measurement of specimens is not significantly affected by the presence of heparinized, sodium citrate, or EDTA anti-coagulants, as shown in a study (Study 1) that included 57 specimens collected into plain, heparinized, sodium citrate, and EDTA vacutainer tubes. By regression: (in cutoff-to-signal ratio)

(Heparin) = 1.08 (Serum) + 0.006
r = 0.99

(NaCitrate) = 1.10 (Serum) + 0.026
r = 0.95

(EDTA) = 0.99 (Serum) + 0.034
r = 0.98

Means:
0.252 (Serum)
0.278 (Heparin)
0.304 (NaCitrate)
0.284 (EDTA)

In another study (Study 2), 18 specimens were collected into plain and sodium citrate vacutainer tubes. By regression: (in cutoff-to-signal ratio)

(NaCitrate) = 1.14 (Serum) – 1.54.
r = 0.97

Means:
13.7 (Serum)
14.1 (NaCitrate)

(See "Alternate Sample Types Graphs".)

Analytical Specificity: Analytical specificity was evaluated at two clinical sites in the United States and one European site. In the United States, serum specimens from 17 subgroups of patients with potentially cross-reacting microorganisms or conditions were tested by IMMULITE Anti-HBc and a commercially available enzyme immunoassay for anti-HBc (Kit A), with the following results:

Sample type	Kit A				Total
	+		–		
	IMMULITE Anti-HBc				
	+	–[1]	+	–[1]	
HAV	18	3	0	26	47
HCV	33	4	0	35	72
HDV	10	1	1	9	21
HEV	0	0	0	9	9
Non-viral liver diseases[2]	10	0	0	44	54
Autoimmune diseases	3	0	0	22	25
CMV	6	1	0	6	13
EBV	2	0	0	17	19
Syphilis	6	0	0	5	11
Toxoplasma	5	0	0	15	20
HSV	11	1	0	35	47
Parvovirus B19	0	4	0	10	14
HIV	34	5	0	12	51
Influenza vaccine recipient	2	1	0	22	25
Transplant recipient	3	1	0	10	14
Dialysis	2	1	0	30	33
Intravenous drug abuser	3	1	0	1	5
Others[3]	2	1	0	3	6
Total	150	24	1	311	486

[1] Includes Indeterminate cases.

[2] Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

[3] Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, eight specimens from antinuclear antibody (ANA) positive patients and 28 specimens from patients with positive rheumatoid factor (RF) were tested by IMMULITE Anti-HBc. IMMULITE Anti-HBc test results were all negative for the eight ANA specimens. IMMULITE Anti-HBc test results were negative for 25/28 RF specimens and positive for 3/28 RF specimens.

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11 Code of Federal Regulations, Title 42, Part 493.1201-493.1285, Subpart K-

Quality Control for Tests of Moderate Complexity, Volume 3. U.S. Government Printing Office; 1993.

Technical Assistance

In the United States, contact DPC's Technical Services department. Tel: 800.372.1782 or 973.927.2828 Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

Tables and Graphs

Precision (ratio)

Site 1

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.27	0.010	3.6%	0.014	5.2%
2	0.43	0.018	4.3%	0.021	4.8%
3	1.03	0.041	4.0%	0.051	4.9%
4	1.78	0.088	4.9%	0.104	5.8%
5	1.95	0.115	5.9%	0.131	6.7%
6	3.34	0.218	6.5%	0.268	8.0%

Site 2

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.25	0.005	2.0%	0.012	4.9%
2	0.43	0.011	2.4%	0.023	5.5%
3	1.03	0.037	3.6%	0.050	4.8%
4	1.94	0.067	3.4%	0.130	6.7%
5	2.11	0.150	7.1%	0.187	8.9%
6	3.80	0.127	3.4%	0.228	6.0%

Site 3

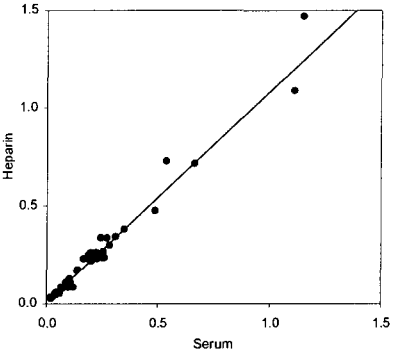
		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.27	0.010	3.9%	0.012	4.6%
2	0.45	0.016	3.6%	0.020	4.5%
3	1.14	0.049	4.3%	0.086	7.6%
4	1.94	0.101	5.2%	0.158	8.1%
5	2.11	0.123	5.8%	0.170	8.1%
6	3.70	0.257	6.9%	0.328	8.9%

Lot-to-Lot and Site-to-Site

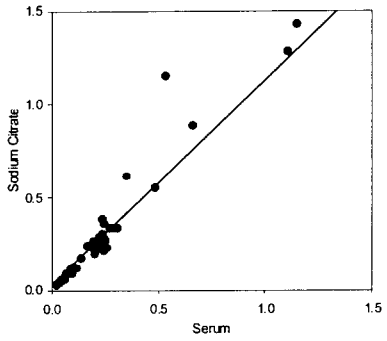
		Lot-to-Lot		Site-to-Site	
	Mean	SD	CV	SD	CV
1	0.34	0.061	18.2%	0.061	18.2%
2	0.56	0.098	17.5%	0.099	17.6%
3	1.02	0.082	8.1%	0.085	8.3%
4	1.65	0.216	13.1%	0.227	13.7%
5	1.91	0.214	11.2%	0.224	11.7%
6	3.00	0.526	17.5%	0.542	18.1%

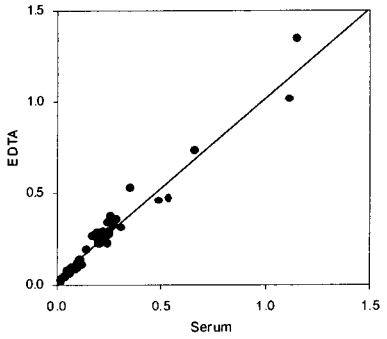
Alternate Sample Types Graphs (ratio)

Study 1



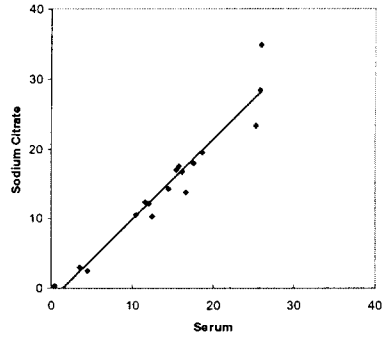
35





Note: One specimen falls outside the range depicted in the graphs above.

Study 2



Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045-5597

2002-07-25 (ISO 8601)

December 9, 2002

PILKHC – 6

IMMULITE® 2000

Anti-HBc

Total Antibodies to Hepatitis B Core Antigen

DPC®

MMULITE® 2000 Anti-HBc

WARNING

This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

Performance characteristics have not been adequately established for use of the IMMULITE 2000 Anti-HBc assay in infants, children, or adolescents.

Federal law restricts this device to sale by or on the order of a physician.

Assay performance characteristics have not been established when the IMMULITE 2000 Anti-HBc assay is used in conjunction with other manufacturers' assays for specific HBV serological markers. Users are responsible for establishing their own performance characteristics.

Intended Use: IMMULITE 2000 Anti-HBc is a solid-phase chemiluminescent enzyme immunoassay designed for use on the IMMULITE 2000 automated immunoassay analyzer for the qualitative detection of total antibodies against hepatitis B core antigen (anti-HBc) in human serum or plasma (EDTA, heparinized, citrate). It is intended for *in vitro* diagnostic use for the presumptive laboratory diagnosis of ongoing or previous hepatitis B virus infection.

Caution: Performance characteristics for the IMMULITE 2000 Anti-HBc were determined in part using archival specimens. Laboratories are advised that they should monitor results using other DPC HBV serological markers or retest questionable specimens with IMMULITE 2000 Anti-HBc or another legally-marketed anti-HBc assay.

Catalog Number: **L2KHC2** (200 tests)

Test Code: **aBC** Color: **Light Gray**

Summary and Explanation

Hepatitis B virus (HBV) is the sole human pathogen in the family of Hepadnaviridiae, a DNA virus, and is found worldwide. Distribution of HBV infection will vary among geographical areas and population groups. Transmission of the virus is due to parenteral contact, through the exchange of blood or blood products, sexual contact, and perinatal spread from mother to newborn.[1,2] Clinical manifestations range from mild asymptomatic infections to severe fulminant hepatitis.[1,2,3] Over 90% of infected adults will have an acute self-limiting infection,[2] with approximately 30% of these individuals developing jaundice, liver enzyme elevations, and symptoms. Recovery occurs without any chronic sequelae.[1,2]

Chronic liver disease, a condition in which infection persists for more than six months, a known sequela of a hepatitis B infection, is usually progressive.[1,2] The risk of developing the chronic carrier state is more likely to follow infection acquired in childhood than as an adult.[4,5] In chronic HBV carriers, there is no evidence of continued hepatic damage,[1,2] however, the infection persists and the carrier maintains the ability to transmit the virus.[2]

Availability of HBV vaccines, and the recommendation of universal immunization for infants and other high-risk persons has aided in the prevention of HBV infections. In addition, treatment with alpha-interferon to relieve symptoms is available. Results have shown positive response to treatment in 40–50% of selected individuals with chronic active hepatitis B.[4,5]

Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. The first marker to appear during the incubation phase is HBsAg, and indicates an ongoing infection with HBV.[1,2,4] IgM and total anti-HBc appears shortly after the appearance of HBsAg, when the patient usually becomes symptomatic, and peaks during the acute phase prior to the appearance of anti-HBs. IgM antibody to the core antigen will decline in uncomplicated acute infection, whereas IgG antibody will persist for years.[4,5] Total anti-HBc may also be elevated in chronic HBV infections.[4]

Presence of IgM and total anti-HBc indicates an ongoing or recent HBV infection. These tests can be used in conjunction with other HBV serological markers for laboratory diagnosis or to rule out HBV infection.

rinciple of the Procedure

IMULITE 2000 Anti-HBc is a solid-ıase, two-step chemiluminescent ızyme immunoassay. The solid phase, a ɔlystyrene bead, is coated with purified ːombinant HbcAg produced in *E. coli* acteria.

he patient specimen and coated bead re added to the Reaction Tube. An lkaline phosphatase-labeled monoclonal nti-HBc antibody is also added to the Reaction Tube. After the wash and ıcubation steps, chemiluminescent ubstrate undergoes hydrolysis in the resence of alkaline phosphatase. MMULITE 2000 Anti-HBc is an antibody ːompetitive assay. The photon output, as neasured by the luminometer, is inversely elated to the presence of antibodies to HBcAg in the sample.

Incubation Cycles: 2 × 30 minutes.

Specimen Collection

Hemolyzed samples may indicate mistreatment of a specimen before receipt by the laboratory; hence, the results should be interpreted with caution.

Centrifuging serum samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

Samples should be thoroughly separated from all cellular material. Failure to do so may lead to falsely elevated results.[9]

The IMMULITE 2000 Anti-HBc assay may be performed on human serum or plasma (heparinized, sodium citrate, or EDTA).

If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.

Specimens containing precipitate may give inconsistent test results. To prevent this problem, such specimens should be clarified prior to assaying. Specimens showing particulate matter or red blood cells may give inconsistent results and should be centrifuged prior to testing (recommended 8,000 to 10,000 RCF × 10 minutes). Specimens, which are not tested within 24 hours, should be removed from the clot or red blood cells.

Do not use heat-inactivated specimens.

Specimens with obvious microbial contamination should not be tested and recollected.

More than two freeze-thaw cycles are not recommended.

Volume Required: 50 µL serum or plasma (heparinized, sodium citrate or EDTA).

Storage: 2 days at room temperature (15°–28°C).[9]
3 days at 2–8°C,[6,9]
For longer storage: at –20°C.[7]

Warnings and Precautions

For *in vitro* diagnostic use.

This assay was designed and validated for use with human serum or EDTA, heparin, and Na Citrate plasma from individual patient specimens.

Reagents: Store at 2–8°C. Dispose of in accordance with applicable laws.

Source materials derived from human blood were tested by FDA recommended test procedures and found nonreactive for syphilis; for antibodies to HIV1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

The Anti-HBc Adjustor, Anti-HBc Low Positive and Anti-HBc Positive Controls contain HBcAg which may be indicative of low levels of HBV. These components have been inactivated by proven, documented methods. However, always handle all controls as if capable of transmitting infectious agents.

Because no test method can offer complete assurance that laboratory specimens do not contain HIV, hepatitis B virus, or other infectious agents, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, *Biosafety in Microbiological and Biomedical Laboratories, 1993.*

ıdium azide, at concentrations less than 1 g/dL, has been added as a ɜservative. On disposal, flush with large lumes of water to prevent the buildup of ıtentially explosive metal azides in lead ıd copper plumbing.

ıemiluminescent Substrate: Avoid ɔntamination and exposure to direct ınlight. (See insert.)

/ater: Use distilled or deionized water for .e wash step and avoid system ɔntamination.

Materials Supplied

he principal components — Bead Pack, :eagent Wedge, and Adjustor — ɜpresent a matched set. Barcodes ncode information about the test, ıcluding expiration dates, component lot ıumbers, and cutoff parameters.

\nti-HBc Bead Pack (L2HC12)
/ith barcode. 200 beads coated with ɔurified recombinant HBcAg manufactured ɔy DPC. Stable at 2–8°C until expiration ıate.

Anti-HBc Reagent Wedge (L2HCA2)
With barcode. 11.5 mL of a protein-based ɔuffer, with preservative. 11.5 mL alkaline phosphatase (bovine calf intestine) conjugated to purified monoclonal murine anti-HBc in buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C until expiration date.

Before use, tear off the top of the label at the perforations without damaging the barcode on the main label. Remove the foil seal from the top of the Reagent Wedge, and snap the sliding cover down into the ramps on the reagent lid.

Anti-HBc Adjustor (LHCR)
2 mL human serum reactive to HBcAg in a buffer, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

Anti-HBc Controls (LHCC1, LHCC2, LHCC3) (may be purchased separately.) Three vials containing 2 mL each. **LHCC1 (Negative Control):** human serum nonreactive to HBcAg, with < 0.1 g/dL sodium azide. **LHCC2, LHCC3 (Low Positive Control, Positive Control):** human serum reactive to HBcAg, with < 0.1 g/dL sodium azide. Stable at 2–8°C for 14 days after opening, or for 6 months (aliquoted) at –20°C.

For the current control ratio ranges, please refer to the Control insert.

Aliquot Labels with barcodes are supplied with the kit, for use with the Adjustors and Controls. Before use, place the appropriate Aliquot Labels on test tubes, so the barcodes can be read by the barcode reader on the IMMULITE 2000.

Kit Components Supplied Separately

L2SUBM: Chemiluminescent Substrate
L2PWSM: Probe Wash
L2KPM: Probe Cleaning Kit
L2RXT: Reaction Tubes (disposable)

Assay Procedure

Note that for optimal performance, it is important to perform all routine maintenance procedures as defined in Section 3 of the IMMULITE 2000 Operator's Manual.

See the IMMULITE 2000 Operator's Manual Section 6 for routine operation procedures (preparation, setup, assay, and quality control) and Section 4 for the adjustment procedure.

Adjustment Interval: 4 weeks. The Anti-HBc Adjustor is used as the cutoff reference to determine the status of a patient specimen.

Quality Control Samples: The controls supplied with the kit should be used as quality control material to monitor assay performance.

The quality control material is in a serum matrix. It may not adequately control the assay for plasma matrix specimens. The user should provide alternate control material for a plasma matrix.

For the current control ratio ranges, please refer to the Control insert.

If control results fall outside the stated range, patient results should not be reported. Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. It is advisable to repeat some or all-patient specimens before reporting results for this run.

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e positive control in the test kit is not to used to quantitate assay sensitivity. ıe positive control is used to verify that ɜ test kit components are capable of :tecting a reactive specimen provided e test procedure has been strictly lhered to.

is recommended that the user refer to *CCLS: Internal Quality Control Testing: rinciples and Definitions* and other ublished guidelines for general quality ɔntrol recommendations and interval for ɜsting.[8,10]

.dditional controls may be tested ccording to guidelines or requirements of ɔcal, state, and/or federal regulations or ccrediting organizations.

'he positive control is used to verify that he test kit components are capable of letecting a reactive specimen provided he test procedure has been strictly ıdhered to.

Calculation of Cutoff and CO/S Ratio:
The Master Cutoff of the assay was determined from representative samples to achieve optimal sensitivity and specificity for the assay.

The cutoff is set equal to the average counts per second of the Adjustor (from the most recent adjustment) multiplied by Curve Parameter 1. (See the "Low Adjustor CPS" and "Curve Parameter 1" fields in the IMMULITE 2000 Kit Information screen, which can be accessed from the menu via Data Entry : Kit Entry.)

Calculation of a cutoff/signal (co/s) ratio is done by using the following formula:

$$\text{CO/S Ratio} = \frac{\text{Mean Adjustor cps} \times \text{P1}}{\text{Sample or Control cps}}$$

Calculation and reporting of qualitative (positive/negative/indeterminate) ratio results are handled automatically by the IMMULITE 2000.

The result for a sample is "indeterminate" if the counts per second for that sample fall within ±15% of the cutoff. The result is reported as "Positive" if the sample's counts are *below* the indeterminate range, and "Negative" if *above* this range.

Interpretation of Results

A result of **"Positive"** (co/s ratio of ≥ 1.15) indicates that anti-HBc antibodies were detected in the sample, which is indicative of either ongoing or previous HBV infection.

A result of **"Negative"** (co/s ratio of < 0.85) indicates that anti-HBc antibodies were not detected in the sample. It is possible the individual is not infected with HBV.

Any result of **"Indeterminate"** (co/s ratio between 0.85 and < 1.15) should be retested. Samples, which still test as "Indeterminate", should be tested by an alternate method, or a second sample should be taken — if possible — within a reasonable period of time (e.g., one week).

For the determination of seroconversion, two sera or plasma (heparinized, sodium citrate or EDTA) samples should be drawn three to four weeks apart, during the acute and convalescent stages of the infection.

It is recommended that the following be included with the report to the physician:

> The following results were obtained with the IMMULITE 2000 Anti-HBc assay. Values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported anti-HBc level cannot be correlated to an endpoint titer.

Limitations

IMMULITE 2000 Anti-HBc assay is limited to the detection of total anti-HBc in human serum or plasma. The presence of total anti-HBc does not constitute a diagnosis of hepatitis B infection but may be indicative of recent and/or past infection by hepatitis B virus. A nonreactive test result does not exclude the possibility of exposure to hepatitis B virus. Levels of total anti-HBc may be undetectable in early infection or when the patient is asymptomatic.

A negative result does not indicate that the patient was not infected with HBV. The patient sample should be tested for the presence of other serological markers, such as HBsAg or anti-HBc IgM.

Testing using alternative methodologies may be warranted if signs, symptoms, and risk factors are indicative of viral hepatitis

ıd other laboratory tests are nonreactive ır the diagnosis of viral hepatitis.

ssay performance characteristics have ot been established for any specimen ıatrices other than serum or heparinized, DTA, or sodium citrate plasma.

he results from this or any other iagnostic kit should be used and ıterpreted only in the context of the ıverall laboratory and clinical picture. Supportive laboratory and clinical ıformation must be used to determinate he HBV disease state. False results may ɔe obtained with any diagnostic test.

Heterophilic antibodies in human serum can react with the immunoglobulins ncluded in the assay components causing ınterference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988:34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of this type of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other laboratory findings.

Expected Values

Demographics and expected prevalence rates for different categories of subjects, each of whom provided one specimen, from three clinical studies, one in the northwestern United States (Study 1), one in the southern United States (Study 4) and one in Europe, are summarized in the following tables.

Apparently Healthy Subjects (Study 1)

IMMULITE 2000 anti-HBc				
Age	Gender	Total	Pos	%
0 - 9	Male	0	0	—
	Female	0	0	—
10 - 19	Male	4	1	25.0%
	Female	5	0	0.0%
20 - 29	Male	7	0	0.0%
	Female	13	1	7.7%
30 - 39	Male	2	0	0.0%
	Female	2	0	0.0%
40 - 49	Male	1	0	0.0%
	Female	1	0	0.0%
50 - 59	Male	0	0	—
	Female	0	0	—
60 - 69	Male	0	0	—
	Female	1	0	0.0%
70 - 79	Male	0	0	—
	Female	0	0	—
80 - 89	Male	0	0	—
	Female	0	0	—
90 - 99	Male	0	0	—
	Female	0	0	—
Totals	Male	14	1	7.1%
	Female	22	1	4.5%
	All	36	2	5.6%

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:her subjects

ubject	Total *n*	Male	Female	Mean Age	Age Range	Pos. by IML 2000 Anti-HBc	
						n	%
pparently ealthy Europe)	1,746	Not available				52	2.9%
re-accination Study 1)	17	8	9	34	21-56	0	0.0%
Pregnant at igh risk[1] Study 1)	3	0	3	33	30-36	3	100.0%
Pregnant at ow risk[1] Study 1)	16	0	16	31	21-42	0	0.0%
Pregnant at ow risk[1] Study 4)	200	0	200	28	17-41	4	2.0%
Pregnant at ow risk[1] (Europe)	22	Not available				0	0.0%

[1] At high risk or low risk of exposure to hepatitis B.

Performance Characteristics

I. Clinical Performance

Four clinical studies with 769 specimens and one comparison study with 139 specimens from a total of 908 subjects were conducted to assess the performance of the IMMULITE 2000 Anti-HBc assay.

The participating subjects each contributed one specimen to the analyses. The specimens were tested by FDA-approved or licensed hepatitis B assays (Reference markers) and IMMULITE 2000 Anti-HBc. All specimens in the analyses were initial test results.

The data were analyzed following the assignment of specimen classification based upon the reactive(+)/ nonreactive(-) patterns for the HBV reference serological markers. Specimen classification was based only on the HBV serological marker results for that particular specimen. No other laboratory or clinical information was used in the disease classification process.

Study 1: Conducted in the northwestern United States, this study included 281 patients, consisting of 138 males and 88 females. Gender for 55 subjects was not reported. These subjects had an average age of 43 years ranging from 21 to 85 years. Distributions of the ethnicity of the subjects are shown in the following table.

Ethnicity	*n*	%
African American	15	5.3%
Caucasian	169	60.1%
Hispanic	2	0.7%
Asian	32	11.4%
Other	3	1.1%
Unknown	60	21.4%
Total	281	100.0%

These subjects included acute and chronic hepatitis B patients, vaccinated individuals, pregnant women, and apparently healthy individuals, and patients with potentially crossreactive substances and medical conditions.

A total of 281 specimens were prospectively (n=92) and retrospectively (n=189) collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 17 unique patterns:

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haracterization ased on single oint specimens	No. of patients	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
\cute	2	+	–	–	–	–	–
\cute	1	+	+	–	–	–	–
\cute	32	+	–	+/-	+	+	–
\cute	34	+	+	+/-	+	–	–
Chronic	2	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	+
Chronic	1	+	–	–	+	–	+
Chronic	1	+	+	+/-	+	–	+
Early Recovery	16	–	–	+/-	+	+	+
Early Recovery	4	–	–	–	+	+	–
Early Recovery	19	–	–	–	+	+/-	–
Early Recovery	1	–	–	+	+	+/-	+
HBV vaccine response	27	–	–	–	–	–	+
Not previously infected	120	–	–	–	–	–	–
Recovered	16	–	–	–	+/-	–	+
Recovered	1	–	+/-	–	+	–	+
Uninterpretable	1	–	+	–	–	–	–

Based on the above classifications the IMMULITE 2000 Anti-HBc results were compared to Kit A, a reference assay for the determination of anti-HBc.

Reference	Kit A						Total
	+			–			
	IML 2000 Anti-HBc						
	+	Ind	–	+	Ind	–	
HB Acute infection	63	0	3	0	0	3	69
HB Chronic infection	7	0	0	0	0	0	7
HB Early recovery	32	0	8	0	0	0	40
HBV Vaccine response	0	0	0	0	0	27	27
Not previously infected	0	0	0	1	0	119	120
Recovered	12	3	2	0	0	0	17
Uninterpretable	0	0	0	0	0	1	1
Total	114	3	13	1	0	150	281

HB Acute Infection
Positive agreement = 95.5% (63/66)
95% CI = 87.3 to 99.1%
Negative agreement = N/A (3/3)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (7/7)
95% CI = 59.0 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Early Recovery
Positive agreement = 80.0% (32/40)
95% CI = 64.4 to 91.0%
Negative agreement = N/A (0/0)
95% CI = N/A

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (27/27)
95% CI = 87.2 to 100.0%

Not previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.2% (119/120)
95% CI = 95.4 to 100.0%

Recovered
Positive agreement = 70.6% (12/17)
95% CI = 44.0 to 89.7%
Negative agreement = N/A (0/0)
95% CI = N/A

Uninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

tal
Positive agreement = 88.7% (114/130)
95% CI = 80.8 to 92.8%
Negative agreement = 99.3% (150/151)
95% CI = 96.4 to 100.0%
Total agreement = 94.0% (264/281)
95% CI = 90.5 to 96.4%

tudy 2: Conducted in the northeastern nited States, this study included 209 atients, consisting of 104 males and 103 emales. Gender for two patients was not eported. These patients had an average ge of 47 years, ranging from newborn to 3 years. Distributions of the ethnicity of ne patients are shown in the following able.

Ethnicity	*n*	%
African American	21	10.0%
Caucasian	101	48.3%
Hispanic	3	1.4%
Asian	6	2.9%
Other	7	3.3%
Unknown	71	34.0%
Total	209	100.0%

Included were patients with potentially cross-reactive substances and medical conditions.

A total of 209 retrospective specimens were collected and tested by FDA-approved or licensed hepatitis B assays for four HBV serological markers (HBsAg, anti-HBc IgM, anti-HBc, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated eight unique patterns:

Characterization based on single point specimens	p ati	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Acute	8	+	–	–	–
Acute	9	+	+/-	+	–
Chronic	2	+	–	+	+
Early recovery	33	–	+/-	+	+
Early recovery	17	–	–	+	–
HBV vaccine response	32	–	–	–	+
Not previously infected	107	–	–	–	–
Uninterpretable	1	+	–	–	+

Based on the above classifications the IMMULITE Anti-HBc results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			–			
	IML 2000 Anti-HBc						
	+	Ind	–	+	Ind	–	
HB Acute infection	9	0	0	0	0	8	17
HB Chronic infection	2	0	0	0	0	0	2
HB Early recovery	45	2	3	0	0	0	50
HBV Vaccine response	0	0	0	0	0	32	32
Not previously infected	0	0	0	0	1	106	107
Uninterpretable	0	0	0	0	0	1	1
Total	56	2	3	0	1	147	209

HB Acute Infection
Positive agreement = 100.0% (9/9)
95% CI = 66.4 to 100.0%
Negative agreement = 100.0% (8/8)
95% CI = 63.1 to 100%

HB Chronic Infection
Positive agreement = N/A (2/2)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

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rly Recovery
Positive agreement = 90.0% (45/50)
95% CI = 78.2 to 96.7%
Negative agreement = N/A (0/0)
95% CI = N/A

BV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (32/32)
95% CI = 89.1 to 100.0%

lot previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 99.1% (106/107)
95% CI = 94.9 to 100.0%

Jninterpretable
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = N/A (1/1)
95% CI = N/A

Total
Positive agreement = 91.8% (56/61)
95% CI = 81.9 to 97.3%
Negative agreement = 99.3% (147/148)
95% CI = 96.3 to 100.0%
Total agreement = 97.1% (203/209)
95% CI = 93.9 to 98.9%

Study 3: Specimens obtained from China were tested in the southern United States. This study included 79 patients and was comprised of 13 females and 55 males (gender for 11 patients was not reported) with an average age of 36 years, ranging from 18 to 82 years. These were prospectively recruited patients from a clinically well-characterized, homogeneous population: acute hepatitis B patients who presented with symptoms typical of acute hepatitis B such as jaundice, persistent fatigue, loss of appetite, pale stools and liver enlargement. Their ALT and AST results were significantly elevated at the time of diagnosis.

A total of 79 specimens were prospectively collected and tested by FDA-approved or licensed hepatitis B assays for six HBV serological markers (HBsAg, HBeAg, anti-HBc IgM, anti-HBc, anti-HBe, and anti-HBs). Characterization based on single point specimens by the reference serological markers demonstrated 11 unique patterns:

Characterization based on single point specimens	n	HBV Reference Markers					
		HBsAg	HBeAg	Anti-HBc IgM	Anti-HBc	Anti-HBe	Anti-HBs
Acute	23	+	–	+/-	+	+	–
Acute	8	+	+/-	+	+	+	–
Acute	1	+	–	+	+/-	–	–
Acute	35	+	+	+/-	+	–	–
Acute	4	+	+	+	+	–	+/-
Chronic	1	+	–	–	+	–	–
Chronic	3	+	+/-	–	+	+	–
Chronic	1	+	+	+/-	+	–	+
Chronic	1	+	+	+	+	+	+
Recovered	1	–	–	–	+/-	–	+
Uninterpretable	1	+	–	+	+	+	+

Based on the above classifications the IMMULITE 2000 Anti-HBc results were compared to Kit A.

Reference Serological Characterization	Kit A						Total
	+			–			
	IML 2000 Anti-HBc						
	+	Ind	–	+	Ind	–	
HB Acute infection	71	0	0	0	0	0	71
HB Chronic infection	6	0	0	0	0	0	6
Recovered	1	0	0	0	0	0	1
Uninterpretable	1	0	0	0	0	0	1
Total	79	0	0	0	0	0	79

HB Acute Infection
Positive agreement = 100.0% (71/71)
95% CI = 94.9 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

HB Chronic Infection
Positive agreement = 100.0% (6/6)
95% CI = 54.1 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A

Recovered
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

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nterpretable
Positive agreement = N/A (1/1)
95% CI = N/A
Negative agreement = N/A (0/0)
95% CI = N/A

al
Positive agreement = 100.0% (79/79)
95% CI = 95.4 to 100.0%
Negative agreement = N/A (0/0)
95% CI = N/A
Total agreement = 100.0% (79/79)
95% CI = 95.4 to 100.0%

tudy 4: Conducted in the southern nited States, this study included :trospectively collected specimens from 00 pregnant subjects. These subjects ad an average age of 28 years, ranging om 17 to 41 years.

\ total of 200 specimens were tested by DA-approved or licensed hepatitis B ıssays for four HBV serological markers HBsAg, Anti-HBc IgM, Anti-HBc, and \nti-HBs). Characterization based on ;ingle point specimens by the reference ;erological markers demonstrated three ınique patterns:

Characterization based on single point specimens	No. of subjects	HBV Reference Markers			
		HBsAg	Anti-HBc IgM	Anti-HBc	Anti-HBs
Early recovery	4	–	+/-	+	+
Early recovery	2	–	–	+	–
HBV vaccine response	42	–	–	–	+
Not previously infected	152	–	–	–	–

Based on the above classifications the IMMULITE 2000 Anti-HBc results were compared to Kit A.

Reference	Kit A						Total
	+			–			
	IML 2000 Anti-HBc						
	+	Ind	–	+	Ind	–	
Early recovery	4	0	2	0	0	0	6
HBV vaccine response	0	0	0	0	0	42	42
Not previously infected	0	0	0	0	0	152	152
Total	4	0	2	0	0	194	200

Early Recovery
Positive agreement = 66.7% (4/6)
95% CI = 22.3 to 95.7%
Negative agreement = N/A (0/0)
95% CI = N/A

HBV Vaccine Response
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (42/42)
95% CI = 91.6 to 100.0%

Not Previously Infected
Positive agreement = N/A (0/0)
95% CI = N/A
Negative agreement = 100.0% (152/152)
95% CI = 97.6 to 100.0%

Total
Positive agreement = 66.7% (4/6)
95% CI = 22.3 to 95.7%
Negative agreement = 100.0% (194/194)
95% CI = 98.1 to 100.0%
Total agreement = 99.0% (198/200)
95% CI = 96.4 to 99.9%

Study 5: In an additional study conducted at Diagnostic Products Corporation, IMMULITE 2000 Anti-HBc was compared to IMMULITE Anti-HBc. Presented below are the comparisons between IMMULITE and IMMULITE 2000 Anti-HBc on a total of 139 specimens.

IML Anti-HBc									
+			Ind			–			
IML 2000 Anti-HBc									
+	Ind	–	+	Ind	–	+	Ind	–	Total
78	0	1	0	0	0	0	0	60	139

Positive agreement = 100.0% (78/78)
95% CI = 95.4 to 100.0%
Negative agreement = 98.4% (60/61)
95% CI = 91.2 to 100.0%
Total agreement = 99.3% (138/139)
95% CI = 96.1 to 100.0%

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Analytical Performance

:e Tables for data *representative* of the ;say's performance. Results are :pressed as a cutoff-to-signal ratio. Jnless otherwise noted, all were :nerated on serum samples collected in :bes without gel barriers or clot-romoting additives.)

.nalytical Sensitivity: Based on studies ith serial dilution of Paul Erlich Institute :ference material Anti-HBc IgG 100)/mL, the analytical sensitivity (last ositive dilution) for IMMULITE 2000 Anti-IBc is 0.40 PEI U/mL.

'he 95% confidence interval at this level 0.40 PEI IU/mL) is 0.36 - 0.44 PEI U/mL.

Precision: Serum samples were assayed n *duplicate* over the course of 20 days, wo runs per day, for a total of 40 runs and 30 replicates at three US sites. (See "Precision" tables.)

Caution: The IMMULITE 2000 Anti-HBc lot-to-lot precision has not been evaluated.

EDTA, *heparin and sodium citrate* samples were assayed in duplicate in three runs on three days at three U.S. sites for three lots of IMMULITE Anti-HBc and one lot of IMMULITE 2000 Anti-HBc. The median total variance of coefficients (EDTA, 4.9%; heparin, 4.4%; sodium citrate, 5.5%) demonstrated that these alternative sample types do not affect the precision of IMMULITE and IMMULITE 2000 Anti-HBc.

Bilirubin: Presence of unconjugated bilirubin in concentrations up to 20 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 10 and 20 mg/dL of bilirubin concentrations. Performance was not established with clinical specimens.

Hemolysis: Presence of hemoglobin in concentrations up to 504 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 168, 252 and 504 mg/dL of hemoglobin concentrations. Performance was not established with clinical specimens.

Lipemia: Presence of lipemia in concentrations up to 3,000 mg/dL has no effect on results, within the precision of the assay. This limit was determined by spiking positive and negative specimens with 500, 1000, 2000 and 3000 mg/dL of lipemia triglycerides. Performance was not established with clinical specimens.

Alternate Sample Types: The measurement of specimens is not significantly affected by the presence of heparinized, sodium citrate, or EDTA anti-coagulants, as shown in a study (Study 1) that included 57 specimens collected into plain, heparinized, sodium citrate, and EDTA vacutainer tubes. By regression: (in cutoff-to-signal ratio)

(Heparin) = 0.98 (Serum) + 0.018
r = 0.99

(NaCitrate) = 1.07 (Serum) + 0.027
r = 0.94

(EDTA) = 0.97 (Serum) + 0.031
r = 0.99

Means:
0.255 (Serum)
0.269 (Heparin)
0.301 (NaCitrate)
0.280 (EDTA)

In another study (Study 2), 18 specimens were collected into plain and sodium citrate vacutainer tubes. By regression: (in cutoff-to-signal ratio)

(NaCitrate) = 0.92 (Serum) + 0.776
r = 0.91

Means:
10.101 (Serum)
10.083 (NaCitrate)

(See "Alternate Sample Types Graphs".)

Analytical Specificity: Analytical specificity was evaluated at two clinical sites in the United States and one European site. In the United States, serum specimens from 17 subgroups of patients with potentially cross-reacting microorganisms or conditions were tested by IMMULITE 2000 Anti-HBc and a commercially available enzyme immunoassay for anti-HBc (Kit A), with the following results:

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	Kit A				
	+		–		
	IMMULITE 2000 Anti-HBc				
ample type	+	–[1]	+	–[1]	Total
AV	18	3	0	26	47
CV	33	4	0	35	72
DV	10	1	1	9	21
EV	0	0	0	9	9
lon-viral liver iseases[2]	10	0	0	44	54
utoimmune iseases	3	0	0	22	25
MV	6	1	0	6	13
BV	2	0	0	17	19
yphilis	6	0	0	5	11
oxoplasma	5	0	0	15	20
SV	11	1	0	35	47
Parvovirus B19	0	4	0	10	14
HIV	34	5	0	12	51
Influenza vaccine recipient	2	1	0	22	25
Transplant recipient	3	1	0	10	14
Dialysis	2	1	0	30	33
Intravenous drug abuser	3	1	0	1	5
Others[3]	2	1	0	3	6
Total	150	24	1	311	486

[1] Includes Indeterminate cases.

[2] Carcinoma, cirrhosis, hepatorenal disease, fatty liver, passive congestion, cholangitis, biliary obstruction, drug induced hepatitis, choledocholithiasis.

[3] Patients with preclampsia, vasculitis, and sarcoidosis.

In the European study, eight specimens from antinuclear antibody (ANA) positive patients were tested by IMMULITE 2000 Anti-HBc. IMMULITE 2000 Anti-HBc test results were negative for all eight ANA specimens.

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ables and Graphs

recision (ratio)

ite 1

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.26	0.006	2.3%	0.008	2.9%
2	0.45	0.012	2.6%	0.016	3.4%
3	1.08	0.039	3.6%	0.045	4.2%
4	1.86	0.063	3.4%	0.085	4.6%
5	1.96	0.105	5.4%	0.115	5.8%
6	3.42	0.107	3.1%	0.279	8.2%

Site 2

		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.25	0.01	2.3%	0.01	4.1%
2	0.44	0.01	2.6%	0.02	5.0%
3	1.04	0.03	2.6%	0.05	4.9%
4	1.78	0.06	3.1%	0.09	4.8%
5	1.90	0.05	2.6%	0.08	4.2%
6	3.28	0.10	2.9%	0.17	5.2%

Site 3

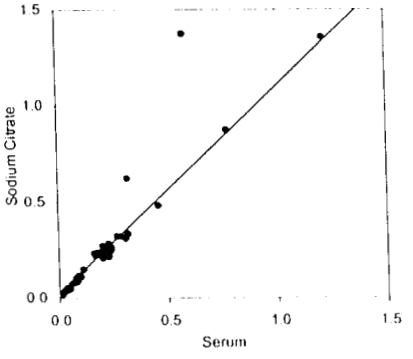
		Intraassay		Total	
	Mean	SD	CV	SD	CV
1	0.26	0.007	2.5%	0.010	3.7%
2	0.45	0.024	5.2%	0.027	6.0%
3	1.07	0.029	2.7%	0.121	11.2%
4	1.93	0.069	3.6%	0.091	4.7%
5	2.08	0.063	3.0%	0.096	4.6%
6	3.59	0.091	2.5%	0.210	5.9%

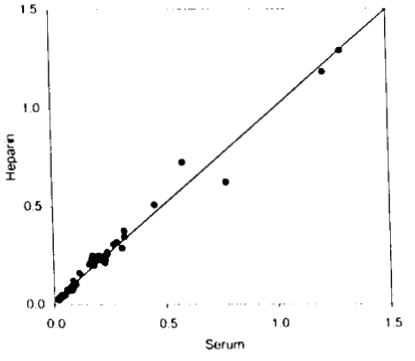
Site-to-Site Precision

		Site-to-Site	
	Mean	SD	CV
1	0.26	0.012	4.7%
2	0.45	0.024	5.3%
3	1.07	0.081	7.6%
4	1.65	0.106	5.7%
5	1.98	0.124	6.3%
6	3.43	0.255	7.4%

Alternate Sample Types Graphs (ratio)

Study 1





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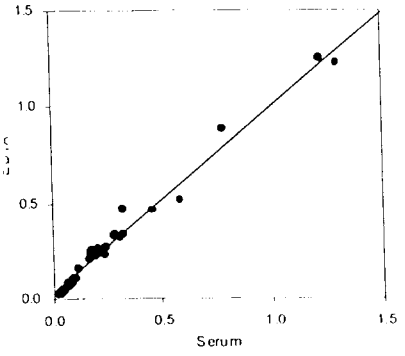
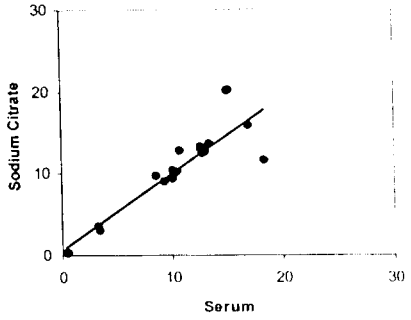


lote: One specimen falls outside the range epicted in the graphs above.

Study 2



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2002-07-25 (ISO 8601)

December 9, 2002

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